Protein-lipid interactions during incubation of whey proteins with autoxidizing lipids

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Introduction

Nowadays, it is well recognized that polyunsaturated fatty acids (**PUFAs**) can provide extensive nutritional and health benefits which has stimulated increased interest towards the **fortification of foods** with oils rich in these particular fatty acids. However, enrichment of food products with such unsaturated fatty acids should be carefully evaluated since they are highly **susceptible to oxidation**. Exposure of proteins to peroxidizing lipids or their secondary breakdown products may induce severe **changes in proteins**. Several amino acids are affected by the secondary **lipid oxidation products**, therefore leading to reductions of their availability. The objective of this study was to characterize changes induced in **whey protein isolate** through interaction with autoxidizing lipids. The impact of the oxidative susceptibility and the oxidation status of the oils on the interaction between the proteins and the autoxidizing lipids were investigated.

Materials and methods

The reaction systems were obtained by mixing 1% (w/v) oil with 2% (w/v) whey protein isolate by means of a vortex for 2 min. The reaction systems in sealed falcon tubes were incubated at 70 °C in the presence of 10 µM $CuSO_4$ solution to stimulate the oxidation and 0.2 g/L NaN_3 to prevent microbial growth. Protein carbonyls were determined after derivatization with 2.4-dinitrophenylhydrazine. Amino acid analysis of the protein samples was carried out after acid or basic hydrolysis using an HPLC system with fluorescent detector. SDS-PAGE was performed under reducing conditions using a stacking gel of 4% and a resolving gel of 15% acrylamide. The fatty acid composition was determined by means of gas chromatography (AOCS Official Method Ce 1b-89).

Results and discussion

The oxidation of whey proteins as assessed via the protein carbonyl content is enhanced by the presence of oils (**Figure 1**). The ability of oils to promote carbonyl formation increases in the order: olive oil < soybean and sunflower oil < soybean oil p-AV 9.9 < soybean oil p-AV 66.2 < fish oil, therefore, dependent on the degree of unsaturation of the fatty acids and initial oxidation degree (**Table 1**).

Lysine, histidine, phenylalanine and arginine were the amino acids the most susceptible to degradation as a result of incubation with fish or highly oxidized soybean oil (**Table 2**).

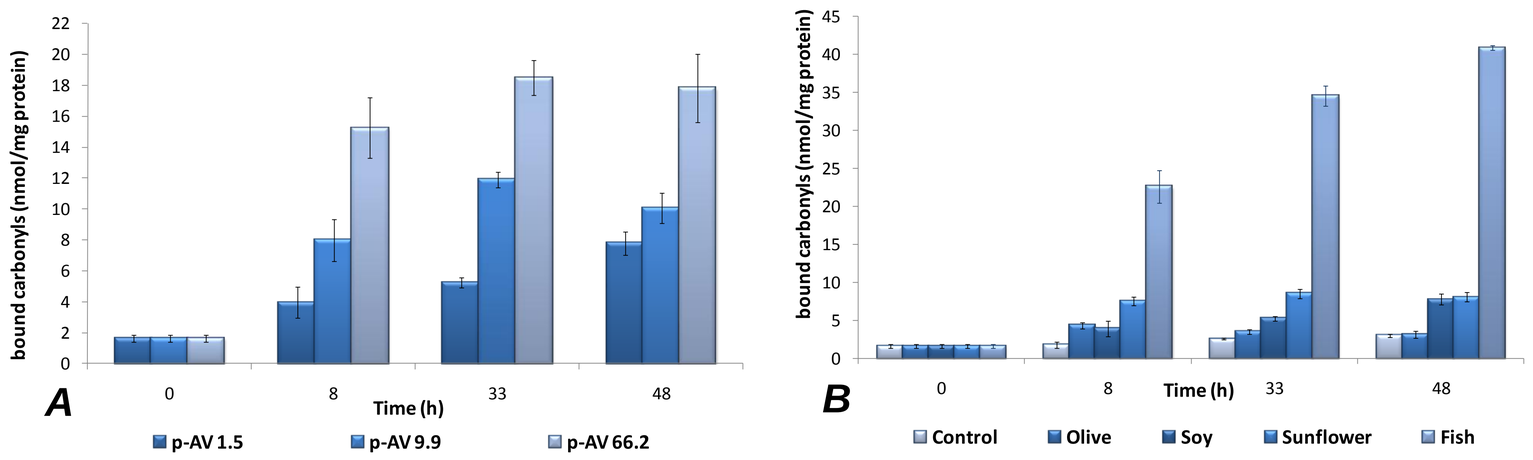

Figure 1 *Effects of oils with different initial oxidation degrees (A) and oils with different unsaturation degree (B) on protein bound carbonyls formation*

Table 1 *Fatty acids composition (g fatty acid per 100g fatty acids) and the initial TOTOX value of the fresh oils used in the reaction systems*

Fatty acids	Olive	Fish	Soybean	Sunflower
18:1	73.2	9.4	24.4	22.0
18:2	7.2	3.9	50.8	57.1
18:3	0.8	1.5	6.2	0.1
18:4	n/d	0.2	n/d	n/d
20:3	n/d	0.4	n/d	n/d
20:5	0.1	18.2	0.2	0.2
22:1	n/d	0.5	n/d	n/d
22:5	0.1	2.1	n/d	n/d
22:6	n/d	12.0	n/d	n/d
TOTOX value	29.32	24.13	5.01	5.58

Table 2 *Effect of highly oxidized soybean oil and fish oil on the amino acid composition of whey protein isolate (mmol amino acid/100g protein), a-significantly different (P<0.05) from the control*

	Control	Soy p-AV 66.2	Fish
Serine	41.02	40.16	39.07[a]
Histidine	10.83	9.45[a]	8.44[a]
Arginine	12.35	11.58[a]	11.07[a]
Tyrosine	17.30	16.72	15.41[a]
Valine	47.66	47.20	44.71[a]
Methionine	14.39	14.45	13.19
Phenylalanine	20.19	19.25[a]	17.71[a]
Lysine	63.96	57.21[a]	48.35[a]
Proline	49.57	48.20	47.23[a]
Tryptophan	5.95	5.61	5.67

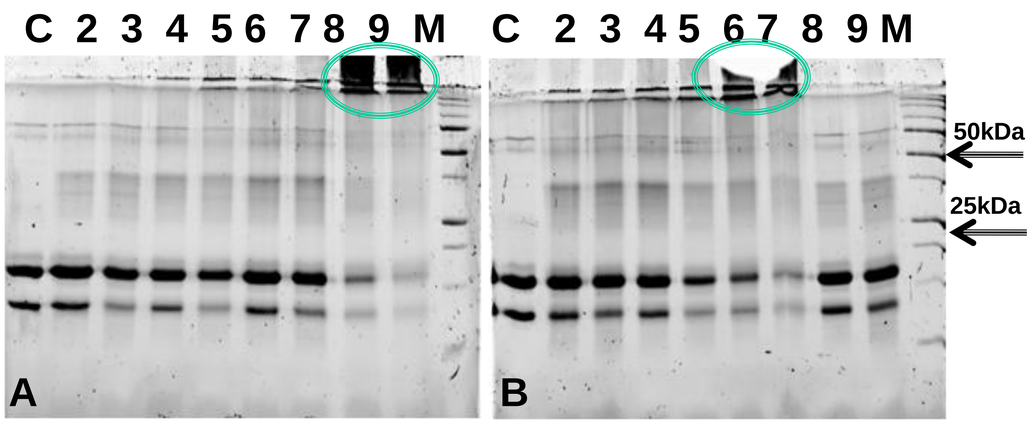

Figure 2 *SDS-PAGE pattern (reducing conditions) of the whey protein isolate incubated with oils: C-control;* ***A*** *- lane 2&3 –24h and 48h with olive oil; lane 4&5 –24h and 48h with sunflower oil; lane 6&7 –24h and 48h with soybean oil; lane 8&9 –24h and 48h with fish oil;* ***B****: lane 2&3 – 24h and 48h with soybean oil p-AV 1.5; lane 4&5 – 24h and 48h with soybean oil p-AV 9.9; lane 6&7 –24h and 48h with soybean oil p-AV 66.2; lane 8&9 – 24h and 48h without oil; M – molecular weight marker.*

The most dramatic changes upon incubation of proteins with lipids was the cross linking of the proteins (**Figure 2**).The aggregates formed are probably due to the interaction of secondary oxidation products with the amino acids either via Michael addition or through formation of Schiff bases. Smearing to the top of the gel indicates aggregate formation with molecular weight of up to 300 kDa, mostly formed upon incubation with oxidized soybean and fish oil.

Conclusions

- *Oxidizing lipids interacted with proteins via reactions between the nucleophilic amino acid residues and secondary oxidation products.*
- *Severe protein aggregation occurred upon incubation of whey proteins with highly unsaturated or oxidized oils.*

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